INTUMESCENCES FORMED AS A RESULT OF CHEMICAL STIMULATION.

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GENERAL DESCRIPTION.

During the winter of 1903, the cauliflowers at the Missouri Botanical Garden were attacked by the crucifer mildew, *Peronospora parasitica*. The plants were sprayed with various copper sprays, to which a small amount of fish glue had been added in order to cause the spray to adhere more firmly to the surface of the leaves. Several days after the spraying, a large number of peculiar wart-like structures began to form on the lower surface of the sprayed leaves. A brief description of these structures was presented in a paper read before the Washington meeting of the Association for the Advancement of Science, and a brief note concerning them was published at that time[24]. It was found that these wart-like formations were in reality large swellings produced in the leaves, which resembled intumescences, a large number of which have been described from time to time on various plants. Owing to pressure of other work, further study of these intumescences had to be dropped at that time, and not until recently, with the reappearance of similar intumescences formed as a result of spraying cauliflowers, was their study taken up again. As stated before, these peculiar warts appeared on the leaves which had been sprayed to stop the mildew. A careful examination was made of all the unsprayed plants in this particular greenhouse, but in no case were such warts found on unsprayed plants.

On plate 25 the photograph of a cauliflower leaf is reproduced, taken from a plant sprayed with copper

[24] Exponential figures refer to the bibliography at end of the paper.

ammonium carbonate, five days after the spraying. It will be noted that the entire lower surface of the leaf is covered with isolated raised warts, which are irregularly scattered over the entire surface of the leaf. These warts are shown somewhat enlarged on plate 26. They form more or less circular, raised knobs of a yellowish color, growing lighter as they grow older, and, after ten days or two weeks, becoming almost white. The individual warts are well defined, rising immediately from the surface of the leaf in a sharply defined circle. Many of the warts grew to a very large size, projecting out from the surface of the leaf, in some cases for one-eighth of an inch.

In the early stages the warts appear as small swellings of the epidermis; the latter, however, was soon broken, or, more correctly, was lifted up on the surface of the growing wart. After six or seven days, a distinct star-like appearance developed under the larger warts. There appeared to be a more or less central body from which lines extended out in a radial direction, giving the appearance of a star-shaped wreath around a central solid body. Reference to plate 26 will show many of the bodies in this condition. At this period the warts were almost uniformly white in color. From the period of this star-like structure, the growth evidently ceased and after several weeks the warts gradually dried up, so that after three weeks or thereabouts they had practically shriveled up. Although both surfaces of the leaves were sprayed, the warts appeared almost entirely on the lower surface of the leaves.

A microscopic examination of the healthy cauliflower leaves shows a well-defined spongy parenchyma layer on the lower surface of the leaves, and a palisade parenchyma, composed of several rows of more or less rectangular cells (plate 27, fig. 1). Sections made through the early stages of the swellings (plate 27, fig. 2) show some of the spongy parenchyma cells enlarged to many times their

size, lifting up the epidermis. In the stage shown in fig. 2, the epidermal cells had shriveled and were practically indistinguishable. Successive stages show an increasing development of giant cells, and a condition after six or seven days as shown in plate 28. Protruding from the surface of the leaf were large masses of enormously elongated very thin-walled cells in a condition of vigorous development. The epidermis had been entirely broken away, and growth was taking place directly out from the center of the mass of tissue and in a lateral direction. The outermost cells, in the stage shown on plate 28, were gradually drying. The giant cells themselves were very thin-walled, closely packed together and practically empty, that is, they were filled with air. In the lower ones, that is those well within the leaf body, very much reduced chlorophyll grains could be found. The whole structure had the appearance of the bodies originally described by Sorauer[26], and called by him intumescences. There were no signs of either fungi or bacteria in the early stages of the wart formation. A study of the drawing shown on plate 28 explains the peculiar outward appearance of the warts, the central body being the cells growing perpendicularly to the surface of the leaves, and the apparent rays constituted by the long hairlike cells pushing out from either side of the central cells. The whitish appearance is explained by the fact that the cells were full of air.

The giant cells originated at first in the outer spongy parenchyma layer, but as the growth progressed, the palisade cells likewise started to enlarge so that in the later stages the entire mesophyll contributed to the formation of the oedematous cells.

Since the formation of the first warts, just described, others have been produced artificially. They agree in most respects with those first formed, with one possible exception. It was found that the size and shape of the warts could be modified at will, depending upon the man-

ner in which the sprays were placed upon the leaf. Where the spray was strictly in the form of small drops, the distinct warts, as shown on plates 25 and 26, were always formed. Where the individual drops ran together making more or less large blotches of the spray, either the entire leaf became oedematous, or there were areas from one-fourth to one-half inch square swelled out, forming enormous massses of giant cells, as shown in plate 31. It will be noted that not only is the leaf lamina affected, but the tissue immediately surrounding and composing the veins of the leaf has likewise been affected. In this case, long lines of oedematous tissue formed on both sides of a vein, breaking out much as in the case of the more circumscribed areas described above.

The formation of the intumescences or oedemas has been known for a long time, both on the leafy and woody parts of a large number of plants. Sorauer[26] designated by the name "Intumescentia" all those structures which appear as small wart-like eruptions on the surface of leaves, generally yellowish in color and which show a more or less unusual stretching of cells. On leaves the swellings generally originate in the mesophyll cells and consist of hypertrophied cells, having a very thin lining of protoplasm, very thin cell walls, and much reduced chlorophyll grains, if any. In the majority of cases a simple stretching of certain cells takes place, but in other cases an actual division and multiplication of cells. This is not confined to the mesophyll, but may also take place with the epidermal cells, as described by Sorauer[33], in the case of intumescences formed on pinks. It will not be necessary to describe in detail the various structural modifications which have been described from time to time for various forms of intumescences on all kinds of plants. The reader is referred to the writings of Sorauer[26–35]; Küster[11,12]; Prillieux[20]; Dale[3]; Trotter[37]; and Atkinson[1].

CAUSE FOR FORMATION.

As to the factors which determine the formation of the intumescences there have been various explanations made from time to time. First among these is the explanation which Sorauer made, when he described these structures, and which he has seen no reason to change in later years. He states[26, 28], "Intumescences are formed when the plants, which for some reason have a reduced assimilatory activity, are brought into conditions which bring about an abnormal turgescence in the cells." "I have therefore regarded the intumescences as a symptom of a disturbance which is caused by an excess of water during a period of low assimilation." Then, again, quoting Sorauer[34]: "So far as can be concluded from my knowledge of the vegetative conditions operative at the time of the formation of these intumescences, all cases point to the conclusion that the affected plants suffered a stimulus because of a heightened temperature, combined with an excess water supply in the tissues, at a time when their assimilatory activity was depressed because of weak illumination, and that as a result of this stimulus, a reaction took place in the form of a stretching of the cell walls at the expense of the cell contents." * * * "Since I have succeeded at other times in producing intumescences experimentally in two cases (*Ficus elastica* and *Impatiens fulva*), one must now without question regard these formations as a symptom of an abnormal elevation of temperature and excessive water supply."

Prillieux[20], Noack[16], Atkinson[1], Trotter[37], and others in general hold with Sorauer that high temperatures combined with a humid atmosphere and abundant water supply are largely responsible for the formation of intumescences.

In addition to the abnormal elevation of temperature

and excessive water supply, the light factor has been considered as having varying influence on the formation of intumescences. Sorauer[34] refers to the fact that weak illumination favors the formation of intumescences because of its lowering the assimilatory activity of the plants. Atkinson[1] holds a similar view, explaining the higher turgescence of the leaves because of the reduced transpiration in poorly lighted greenhouses. He furthermore refers to the fact "that the lack of light not only favors the accumulation of water, * * * but it prevents the plants from building up strong tissue." He even goes so far as to state that "possibly artificial light might be used to advantage" (pg. 108). Trotter[37] likewise says that semi-darkness favors the development of the intumescences. Küster[11], in producing artificial intumescences on poplar leaves, found that intumescences were formed on the leaves which were exposed to intense light, particularly on the sides lying on the water, but that in general intumescences were formed both in the dark and in the light. Too strong light according to him, however, stops the formation, possibly on account of the reduced transpiration. He concludes by saying: "that the formation of intumescences is due to a specific action of light, I am inclined to doubt; it seems more likely that in the bright light, even in the closed dishes, the upper (actual, and not morphologically upper) surface transpired actively, and that therefore no intumescences were formed on that side."

As against the claims that darkness favors the formation of intumescences a number of recent investigations seems to indicate that light is extremely necessary to their formation. Dale[3] finds that white light, or in particular the yellow or red rays, are absolutely essential to the development of the intumescences. In the dark, or in green or blue light, no intumescences were formed on *Hibiscus*. Viala and Pacottet[38], in a description of the intumes-

cences formed on grape leaves, in greenhouses, make the very definite statement, "We have established the fact by means of direct experiments that the intumescences are caused by an excess of light in a humid atmosphere." * * * "The light is the predominating factor." * * * "It is only during periods of the most brilliant illumination and directly under the glass of the houses, that the intumescences form in quantity. One does not observe them in the same greenhouse on leaves which are in a diffuse light, or in the shade." They explain this apparent action of the light by stating that the oedematous leaves protect themselves like succulent plants by the formation of a false palisade tissue "against chlorovaporization and an excessive transpiration," which latter is accentuated by the direct action of the sunlight, both because of light and heat radiation. They recommend preventing the formation of intumescences by shading the glass of the houses.

Observations made in the greenhouse of the Missouri Botanical Garden during the present season on grape vines which were covered with these intumescences, fully bear out the observations made by Viala and Pacottet. The intumescences were found only on the leaves immediately under the glass, while all the leaves in the shade were free from them.

The above quotations seem to leave the question as to what the influence of light on the formation of these intumescences is, in a rather indefinite condition, and it is evident that a good deal of careful experimenting will have to be done to determine the exact nature of the light stimulus, should there be such a one.

Practically all of the explanations made for the production of intumescences have, as has been shown, been caused by conditions of excessive humidity and temperature, possibly with the aid of light. The formation of intumescences because of chemical stimulation has been referred to but twice so far as known. Sorauer[31] in describing the

effects of spraying potatoes with Bordeaux mixture and sulfo-stearite (?) of copper, notes the formation of some burnt spots on the leaves at points where the copper solution stuck to the leaves. Not infrequently the entire upper surface of the leaves turned slightly brown. Under these spots the palisade parenchyma cells had stretched to an abnormal extent and had formed wart-like bodies on the surface of the leaves. In extreme cases this stretching of the cells was so large that the epidermis was torn, exposing the underlying giant cells. These generally had very little chlorophyll and were frequently divided by cross walls. It should be noted, however, that Sorauer found these oedemas not only on the plants which were sprayed with the copper, but also on the unsprayed plants. In all cases the epidermal cells overlying the enlarged mesophyll were apparently dead and brown and corky. Sorauer calls attention to the fact that it has been shown for other plants that such intumescences arise when the assimilatory activity of the leaves has been reduced, combined with a large water absorption and high temperature, and he states that the formation of these intumescences in the leaves of the potato is to be regarded as a sign of lessened assimilatory activity. He states, furthermore, that this is a fairly regular appearance during later stages of development on the leaves, referring in these cases to plants which have not been sprayed. Referring to the leaves sprayed with copper salts he says, "Where this appearance (intumescences) occurs on leaves which are still at the height of their development it must be due to the effect of the copper, and the intumescences must be regarded as resulting from a condition of high turgescence existing in the leaf cells, which would not equalize itself throughout the leaf lamina because of the death of certain epidermal areas." He regards the frequent and numerous appearance of these oedemas as a proof that the copper salts inhibit to a certain extent the normal development of the leaf.

The fact that Sorauer found these oedemas in both sprayed and unsprayed leaves would lead one to suggest that the formation of the oedema in the potato leaves was not always directly connected with the spraying with copper salts.

Küster[11, 12], discussing the formation of intumescences, expressed the belief that intumescences might be formed as a result of the introduction of nutrient or poisonous substances. He refers to the relationship existing between intumescences and the galls produced on poplar leaves by *Harmandia tremulae* and *H. globuli*. He found that the leaves affected with the galls had a tendency to form excessively vigorous intumescences around the base of the galls when placed on the surface of a culture medium. Küster is not certain whether this excessive development of intumescences is due to the action of the gall forming poison, or whether it is connected with the unusual concentration of nutritive substances which form as a result of the gall formation.

The production of giant cells such as form in intumescences has been noted in several instances in connection with insect galls. Woods[39] in describing changes brought about in the leaves of carnations, as a result of punctures by aphids, says that "the cells of diseased spots were found to be much larger than normal, and thin walled and oedematous." In the early stages of the disease, the chloroplasts were undeveloped or smaller than in the healthy cells, and were colorless or yellow. Other instances of the formation of giant cells in insect galls have been reported from time to time[9, 13].

Summing up our present knowledge of the cause for the formation of giant cells, especially in intumescences, it may be said that these are generally formed because of the presence of excessive moisture and heat in enclosed houses, aided possibly by the action of light, and that they have

been reported once as being due to the probable action of copper sulphate.

In the case of the intumescences formed on cauliflower in the Missouri Botanical Garden, the factors of moisture, heat and light played absolutely no part whatever. As stated above, in no case were any intumescences formed except as the immediate result of spraying with various fungicides. The first intumescences which formed on the cauliflower leaves, showed on those leaves which had been sprayed with a standard solution of copper ammonium carbonate, to which a certain amount of fish glue had been added. No intumescences appeared on the leaves sprayed with Bordeaux mixture or potassium sulphide, the other sprays used at the time. It would appear from this that the intumescences must have been caused either by the copper ammonium carbonate or by the glue, or by some substance formed as a result of the combination of copper ammonium carbonate and the glue. In order to test which one of the substances brought about the formation of the intumescences a number of thrifty cauliflower plants were selected which were sprayed as follows: —

Plant No. 1. Four leaves sprayed with fish glue in its concentrated form.

Plant No. 2. Two leaves sprayed with fish glue diluted 50% with water.

Plant No. 3. Four leaves sprayed with Bordeaux mixture, 6 lbs. $CuSO_4$, 4 lbs. lime and 50 gallons water.

Plant No. 4. Four leaves sprayed with Bordeaux mixture to which about 1/3 glue was added.

Plant No. 5. Four leaves sprayed with ammonium copper carbonate.

Plant No. 6. Four leaves sprayed with ammonium copper carbonate to which about 1/3 glue was added.

The plants were sprayed about twelve o'clock and in the bright sun. The spots of Bordeaux mixture and ammonium copper carbonate dried in about two hours. Five days after the spraying the following condition was noted: —

Plant No. 1. With glue. The tissue under the glue spots had shrunken and died, both on the upper and lower surface of the leaves. There was no sign of oedematous tissue under or near any of the spots.

Plant No. 2. 50 per cent. of glue. The same condition as noted for the first plant was true here.

Plant No. 3. The plant sprayed with Bordeaux mixture showed no change whatever under the sprayed spots.

Plant No. 4. The plants sprayed with Bordeaux mixture and glue showed no change whatever.

Plant No. 5. Leaves sprayed with ammonium copper carbonate showed a discoloration of the leaves, not only under the immediate spot where the drops of solution struck the leaves, but extending considerably beyond these spots. On the lower side of two of the sprayed leaves, small warts were forming in considerable numbers, some of which had already broken through the epidermis.

Plant No. 6. The same condition as noted for plant No. 5 was true in this case. That is, on most of the leaves which were sprayed warts were forming on the lower surface.

It appeared from this experiment that the intumescences were not formed by the glue, or by the Bordeaux or by the combination of glue and Bordeaux mixture. It furthermore appeared that the glue was not a necessary element to their formation, when mixed with ammonium copper carbonate, for the ammonium copper carbonate produced the intumescences without the glue addition. The plants were examined again after 11 days and no change whatever had taken place, except in the case of plants sprayed with ammonium copper carbonate and its glue addition. By that time the plants which had been sprayed with ammonium copper carbonate alone showed a great number of swellings, particularly on the lower surface of the leaves. Where the spray struck the leaves in large blotches, the eruptions took place chiefly around the edges of the sprayed spot. In the case of the plants to which the glue had been added, the eruptions were practically confined to the small spots, for with the glue addition the spray came out very fine. The eruptions in this case were generally not more than two to three millimetres in width.

Several experiments were then made with ammonium copper carbonate alone and it was found in practically every instance that within five or six days large numbers of intumescences could be produced as a result of the

spray. The conclusion may therefore safely be drawn that the spray with the standard solution of copper ammonium carbonate is capable of producing typical intumescences on plants, such as are usually formed as a result of excessive moisture absorption at a time when the plant is in a condition of weakened vitality. The demonstration was a very striking one because in no case did any of the plants, except the sprayed ones, produce a single intumescence. Both old and young leaves were sprayed and it was found that the young leaves had as a rule produced a larger number of intumescences and bigger ones. The lower surface of the leaves produced larger numbers than did the upper surface. From the result of Dale's experiments[3], in which it was shown that the intumescences sometimes form under a stoma, it was thought possible that the more frequent occurrence of the intumescences on the lower surface of the leaves of the cauliflower might have some connection with the unequal distribution of the stomata on the upper and lower surface of the leaves. It was found, however, although the number of stomata on the lower side of the leaf is about twice that on the upper side, that the location of the intumescences bore no relation to the position of the stomata.

Having shown that the intumescences were formed as a direct result of the application of ammonium copper carbonate, the next point to be determined was whether the active agent in the formation of the intumescences was the ammonia or the copper. The copper carbonate solution used in the test was made according to the standard method, as follows: 5 oz. copper carbonate, dissolved in a mixture of 3 pints ammonia to 50 gallons of water. The solution was found to contain 1.57% ammonia. Some of the solution used in the first experiment was neutralized with hydrochloric acid and this neutral solution was used in spraying a number of plants. In every instance the tissue underlying the spots formed where the solution dried, died

within three days. The influence of the solution was evidently a very caustic one because the injurious effect was made evident within twenty-four hours after the spraying. The leaf tissue was killed throughout its entire thickness, as shown on the upper side of the leaves, where the surface appeared shrunken and discolored. Grape leaves sprayed at the same time with this solution likewise had a browned appearance after twenty-four hours, and in a very few days all of the tissue struck by the spray was killed.

A third series of experiments consisted in spraying a number of thrifty cauliflower plants with various solutions of copper salts, without any ammonia addition, and another set of plants with ammonia solution and ammonium carbonate solution. The plants sprayed were as follows:—

Series No. 1. Sprayed with ammonium copper carbonate, using the same solution as in the previous experiments.

Series No. 2. Sprayed with copper nitrate solution (15 gr. copper nitrate [$Cu(NO_3)_2 + 3H_2O$] per litre).

Series No. 3. Sprayed with copper acetate solution (12 gr. per litre).

Series No. 4. Sprayed with copper chloride solution (10 gr. per litre, the copper being equivalent of the amount in Bordeaux mixture).

Series No. 5. Sprayed with copper sulphate ($CuSO_4 + H_2O$, 15 gr. per litre).

The ammonia used was an aqueous solution containing 41.88% total ammonia as hydrate. Based upon this determination various strengths of solution were made.

Series No. 6. Were sprayed with 10% ammonia (that is, 4.188% actual ammonia).

Series No. 7. Sprayed with 2% ammonia (that is, .837% actual ammonia).

Series No. 8. Was sprayed with an aqueous solution of ammonium carbonate $(NH_4)_2CO_3$, 5% solution.

After four days these plants showed the following conditions: —

Series No. 1. Ammonium copper carbonate. Younger sprayed leaves show distinct eruptions on the lower surface, which were strictly confined to the places where the spray struck the surface of the leaf. The eruptions were in every way characteristic intumescences. Where the spray struck the leaf in any quantity the eruptions were in large masses.

Series No. 2. Copper nitrate. Where the copper nitrate struck the leaves the tissue underneath was killed entirely (plate 28, fig. 1). Where infinitesimally small drops struck the surface of the leaf, eruptions the size of a pin began to form.

Series No. 3. Copper acetate. The copper acetate solution practically killed the leaf surface wherever it struck in any quantity. Where very small drops struck the surface, exceedingly minute eruptions began to form.

Series No. 4. The copper chloride solution killed the leaf surface wherever the solution struck the leaf (plate 30, fig. 2). Where the spray stuck to the leaves in small drops eruptions started. This was particularly true of the very young leaves.

Series No. 5. Copper sulphate. Plants sprayed with copper sulphate had practically the same appearance as those sprayed with copper chloride and copper nitrate. The sprayed areas were practically killed where the spraying solution had adhered, except where very small quantities of spray stuck to the leaf. It was very striking that not only were the areas immediately touched by the spray killed, but all adjacent areas, as if there had been a decided diffusion of the salt through the leaves (plate 30, fig 1).

Series No. 6. 10% ammonia. Leaves sprayed with 10% ammonia were killed outright in the region touched by the spray (plate 29, fig. 2). Very definite circumscribed spots as shown in the figure were formed. Absolutely no sign of any eruptions.

Series No. 7. 2% ammonia. After four days there was practically no sign of any change in the leaves sprayed with 2% ammonia. Several days later, however, most decided eruptions formed on these leaves and developed in the characteristic manner.

Series No. 8. Ammonium carbonate. The younger leaves showed decided eruptions in blotches wherever the spray struck the leaf. These were beginning to stretch through the epidermis much as the small warts did with the ammonium copper carbonate.

The sprayed plants were examined daily for a period of two weeks and the general conclusions drawn were as follows: —

Plants sprayed with the copper salts without the addition of ammonia were generally killed where the spray struck the leaf areas in any quantity. Where very minute quantities of the spray touched the leaves, eruptions formed. The plants sprayed with ammonia were killed when the stronger solution was used, but the weak solution produced characteristic eruptions. The ammonium copper carbonate produced decided eruptions wherever used.

The results of these experiments seem to indicate that the formation of the intumescences on the cauliflower leaves might have been due to either the copper or the ammonia in the ammonium copper carbonate solution. The ammonium carbonate solution was selected for the experiments because ammonium carbonate gives off ammonia very slowly when exposed to the air. The experiment just described was repeated several times, using the ammonia in various concentrations, and ammonium carbonate, and in nearly every instance eruptions of a characteristic form were produced. In applying the spray to the leaves, a glass atomizer was used in which the copper solutions had likewise been placed for the first tests. Although several days had elapsed since using the copper solutions in the atomizer and although it was thoroughly cleaned and washed, it was thought barely possible that some copper might have been retained by the walls of the glass vessel, as described by Nägeli[15]. On that account the tests were repeated, using ammonia and ammonium carbonate, and this time the sprays were applied to the leaves by means of brushes, so that all chances for the possible contamination with copper were avoided. In this last series of experiments all of the plants sprayed with ammonium copper carbonate, two of the plants sprayed with ammonium carbonate and two of the plants sprayed with 2% ammonia developed intumescences. Two of the plants sprayed with 2% ammonia showed only discolored spots. Of the plants sprayed with ammonium carbonate, one only showed discolored spots. While several of the sprayed spots undoubtedly formed intumescences as a result of spraying with ammonium carbonate or ammonia, owing to the negative results of several plants it will not be possible without further tests to definitely ascribe the formation of these intumescences to the ammonia.

At the same time that the cauliflower leaves were

sprayed, experiments were made with various other plants, including beets, grapes, radishes, violets and a species of *Mesembryanthemum*. On none of these leaves were any intumescences formed, such as appeared on the cauliflower leaves.

One of the most striking results of the spraying with the copper salts was the different effect of the solution when sprayed onto the leaves in large quantities and in very minute drops. When in quantity, the copper solution almost invariably killed the entire underlying tissue completely, whereas in very fine drops the spray evidently stimulated the leaf tissue, forming intumescences. This result was similar to the one obtained by Dandeno[4], who found that "dilute solutions applied in drops stimulated the leaf tissue in a ring, whereas if the solutions are concentrated, the entire area covered by the drop is affected."

Summarizing the results obtained from spraying, it may be stated: First, that spraying uninjured cauliflower leaves with dilute solutions of copper ammonium carbonate, or with other copper salts, results in the formation of marked intumescences which are particularly prominent on the lower side of the leaves. Second, spraying leaves with dilute solutions of copper chloride, copper nitrate, copper sulphate, and copper acetate, kills the leaf tissue where the spray strikes the same in any quantity. Where very minute drops of the spray struck the leaf surface, small intumescences formed much as they do with copper ammonium carbonate. Third, these intumescences are formed as a direct result of the spraying, and they have no connection whatever with conditions which have usually prevailed in the formation of intumescences, such as excessive water supply and high temperature. In other words the intumescences form as a result of a direct chemical stimulation. Fourth, cauliflower leaves are particularly susceptible to such stimulation. Leaves of the grape, violet, radish, beet and *Mesembryanthemum* did not react.

The effects of spraying with various solutions on leaves, have been made the subject of numerous investigations, particularly with reference to the action of various fungicides on growing leaves. The apparent stimulating effects which various copper solutions in particular have upon crops, have become generally recognized. Rumm[22] ascribed the more vigorous appearance of leaves sprayed with Bordeaux mixture, to a stimulation exerted by the copper salt. He, however, left out of consideration the possible influence of the lime. Frank and Krüger[27], finding that the development of potatoes was hastened by spraying with Bordeaux mixture, as evidenced by healthier leaves, more chlorphyll, etc., came to the conclusion that the copper was the active agent in stimulating the plants. They found no proof that the copper entered into the cells and regarded the stimulating activity of the Bordeaux mixture as due to chemotactic stimulation, exerted by the copper, much as did Rumm[22]. Galloway and Woods[8], experimenting on the influence of Bordeaux mixture on potatoes, came to the conclusion that while the spraying with Bordeaux without question stimulated the growth of certain plants, the beneficial effect may be in part due to the presence of the mixture in the soil as well as on the leaves. Sebelieu[25], Woods and Bartlett[40] and Pierce[19], among many others, testify to the beneficial effects of spraying with Bordeaux mixture; in each case ascribing the beneficial effects to the action of the spray upon the leaves. The general consensus of opinion at this day seems to be that either the copper hydrate or lime, or both acting together, in some way stimulates the leaves of various plants to a healthier activity.

On the other hand, it has been found now and then that copper sulphate sprays exerted a more or less harmful influence upon the development of sprayed leaves. Duggar[5, 6] reports the production of shot-hole injuries on leaves when sprayed with Bordeaux mixture. He also obtained similar

results with formalin, picric acid and corrosive sublimate. Dandeno in a recent paper[4] describes at considerable length a number of experiments which he performed to test the effects of water and solutions of various salts on foliage leaves. He finds "that pure water *may be* absorbed." Nutrient solutions (?) he finds are absorbed when sprayed upon the growing leaf, as evidenced by the increased dry weight. The part of his paper which has a particular bearing upon the present subject deals with the effect of "a solution applied to the leaf surface." He experimented with a m/4 strength solution of $MgCl_2$, $ZnSO_4$, Na_2CO_3, KBr, $NaHCO_3$, K_3PO_4, $Na\bar{A}$, $KClO_3$, KI, and with a m/56 solution of $CuSO_4$, and nutrient solution. The plants used were *Ampelopsis*. He likewise experimented with $CaH_2(CO_3)_2$ and $Ca(OH)_2$ on leaves of *Begonia*, *Primula obconica*, *P. stellata*, *Pelargonium* and *Heliotropium*. As a result of his tests he finds that some of the solutions produced yellow and some brown spots on the surface of the leaves, that many of the salts are absorbed through both surfaces of the leaves, as indicated by the absence of any distinct spot when the water evaporates. The solutions were absorbed more easily by the lower side than by the upper side.

Only in one case did he notice any structural changes in the leaves. In spraying tobacco leaves with caustic soda, 1% and 2½% strength, peculiar spots were formed. The tissues in these spots were dead in the center and surrounded by a ring of expanded spongy parenchyma cells which contained larger and more numerous chlorophyll grains than the normal tissue of the leaves. He suggests that the increase in the number and size of the chloroplasts might be due to a stimulus exerted by the caustic solution and refers to the results of Griffon, Ewart and Mayer and others, who found that potassium nitrate and potassium carbonate affected the chloroplasts in some way, resulting in increased dimensions and, in general, in a deeper green

color of the leaves. Dandeno concluded from his research that "the alkali kills the tissue in direct contact with the irritant; it stimulates the abnormal development of the tissue immediately around the spot."

Aside from the stimulating activity noted above, there have been frequent references to the stimulating effect of various salts on both higher and lower plants. Small additions of zinc or magnesium have frequently been found to increase the crop of spores in fungi[17, 21]. The results of all of these investigations show that various dilute solutions, generally of inorganic salts, may have a beneficial effect upon growing cells up to a certain concentration. This beneficial effect is evidenced in more vigorous assimilatory activity and increased production of chloroplasts, and in some cases in an increased cell production. After reaching a certain concentration, the action of the salt has usually been that of an irritant poison, which usually resulted in the temporary disabling and generally in the death of the cells affected. The only cases where the action of a probable poison resulted in growth at all comparable to the intumescences above described, is in connection with various insect galls. It is probably true that the formation of insect galls is due to some chemical influences exerted either by the parent insect, the egg or the larvae, and as a result of these influences, oedematous cells are sometimes produced, as described by Küstermacher[13], Küster[10], and Woods[39].

In none of the effects noticed as a result of the action of chemicals on leaves, with the possible exception of insect galls, has any change in the tissues, such as the oedemas described, been noted. Where copper salts were absorbed there was either a stimulus in the way of increasing production of cell contents, or increased assimilation, or where stronger solutions were used, the cells were destroyed entirely. In the case of the cauliflower intumescences, there is a very evident stimulation resulting from the appli-

cation of various salts, which shows itself in the development of giant cells, or in a state of excessive turgescence. The salts of copper and ammonia must be regarded as exerting a poisonous effect, that is they produce a functional derangement in the organism which sooner or later results in death of the affected parts. The action of poison in accelerating growth has been frequently noted, as for instance in the case of chloroform or ether which may start into growth, or accelerate growth of, resting organs. "Poisons," says Pfeffer[18], "like all other chemical influences, cause not only a retardation but also accelerate either temporarily or permanently, the sum total of the activities or special functions."

In the case of the leaves of the cauliflower sprayed with copper and ammonium salts, a very definite reaction took place which showed itself soon after the application of the sprays in the very much increased turgidity of the spongy parenchyma underlying the sprayed spots. This increased turgidity may have been due to one of two causes. A reaction may have taken place between the stimulating salt and certain elements of the protoplast, resulting in the formation of compounds within the protoplast having enormously high osmotic coefficients. In order to have a poisonous effect, as indicated by Pfeffer, most poisons must be not only actively in contact with the protoplasts, but they must actually enter into contact with the same. "The action of the poisonous heavy metals is probably due to their union with bodies which form parts of developing proteid substances" (Pfeffer[23]). It is easily conceivable that the copper salts might have penetrated into the leaf tissue, resulting in the formation of a compound as suggested. The second explanation is that possibly the various salts exerted some stimulating influence on the protoplasts, of a character not understood, which resulted in a reaction causing the production of a large amount of organic acids, which would explain the increased turgidity of the

cells. As indicated by tests made with 2% gum guaiac solution, the oedematous cells were universally very much richer in oxidizing enzymes than were the healthy cells. What possible effect the production of such oxidizing enzymes will have, will for, the present have to remain undetermined. Sorauer[31] explains that the intumescences formed as a result of spraying with Bordeaux mixture on potatoes, were due to a tendency on the part of the plant to equalize the water present within the cells as a result of the death of the overlying epidermal cells; but this can hardly hold in the case of the cauliflower intumescences because these formed within the leaf tissue before there were any signs of death on the part of the epidermal cells.

Whatever the actual relationship between the sprayed salts and the protoplast may be, one is obviously dealing with a condition of chemical stimulation of living cells hitherto unrecorded. Detailed tests on a large scale are at present in progress, and it is expected that these experiments may throw further light on this peculiar formation.

SUMMARY.

The results of the present investigation may be briefly stated as follows; —

1. Cauliflower plants sprayed with copper ammonium carbonate produced large numbers of intumescences as a direct result of the spraying.
2. Similar intumescences were produced by means of weak solutions of copper chloride, copper acetate, copper nitrate and copper sulphate when sprayed in very fine drops on the surface of the leaves.
3. The intumescences were formed in larger numbers on the lower surface of the leaves than on the upper surface of the leaves.
4. Intumescences were formed independent of soil or atmospheric conditions, so that the heat and water supply had nothing to do with their formation.
5. Intumescences must be regarded as a result of the stimulating activity of chemical poisons, sprayed upon the leaf in weak solutions.
6. The stimulating activity exerted is probably due to the formation of compounds within the cells of high osmotic tensions, these com-

pounds being either compounds formed by the copper salts with parts of the protoplast, or compounds formed as a result of a stimulus exerted, as evidenced by the presence of large amounts of oxidizing enzymes as a result of an indirect stimulus exerted by the salts sprayed upon the leaf surface.

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DESCRIPTION OF PLATES.

Plate 25. — Photograph showing the under surface of cauliflower leaf sprayed with copper ammonium carbonate. Appearance five days after spraying.

Plate 26. — Photograph showing a small portion of cauliflower leaf sprayed with copper ammonium carbonate, five days after spraying. Approximately natural size.

Plate 27. — Sections of cauliflower leaf. 1, Section through a healthy leaf, showing the shape of the cells. The cell contents have been omitted for the sake of clearness. 2, Section of leaf through a growing intumescence, showing the enlarged spongy parenchyma cells.

Plate 28. — Section through a fully formed intumescence on cauliflower leaf, five days after spraying with copper ammonium carbonate.

Plate 29. — 1, Cauliflower leaf sprayed with copper nitrate, showing the dead areas, wherever the spray struck the leaf. 2, Cauliflower leaf sprayed with 10 per cent. ammonia, showing the killed areas of the leaf.

Plate 30. — 1, Cauliflower leaf sprayed with copper sulphate, showing the dead area wherever spray struck the leaf. 2, Cauliflower leaf sprayed with copper chloride, showing dead area where the spray struck the leaf.

Plate 31. — Cauliflower leaf sprayed with copper ammonium carbonate, showing how the leaf was killed where the spray struck in large quantities, with intumescences where small drops struck.

INTUMESCENCES OF CAULIFLOWER.

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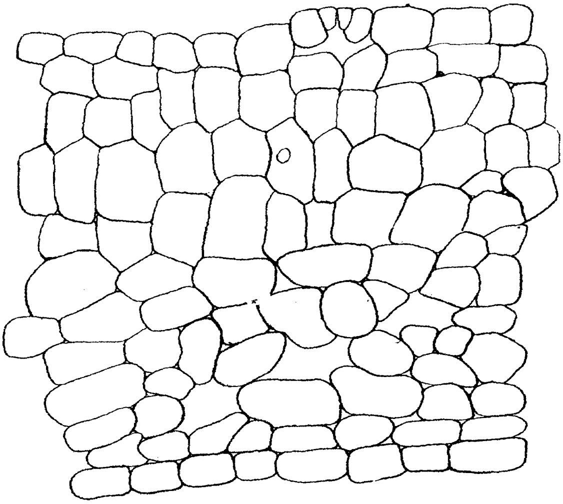

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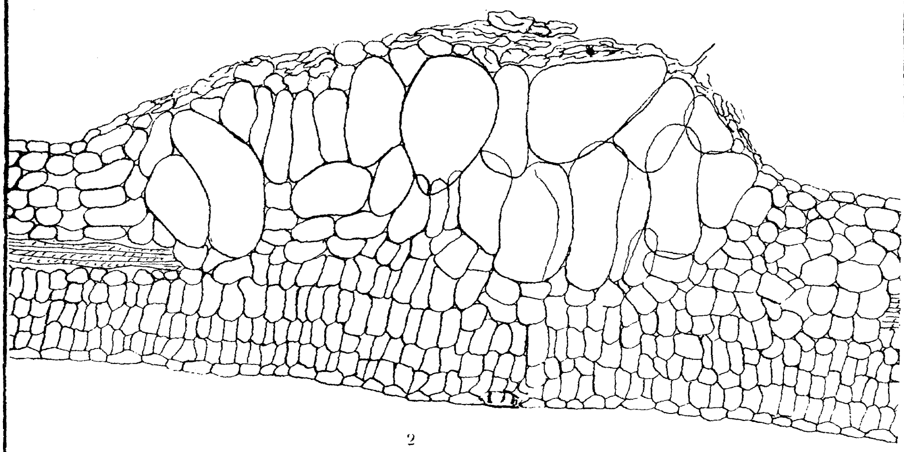

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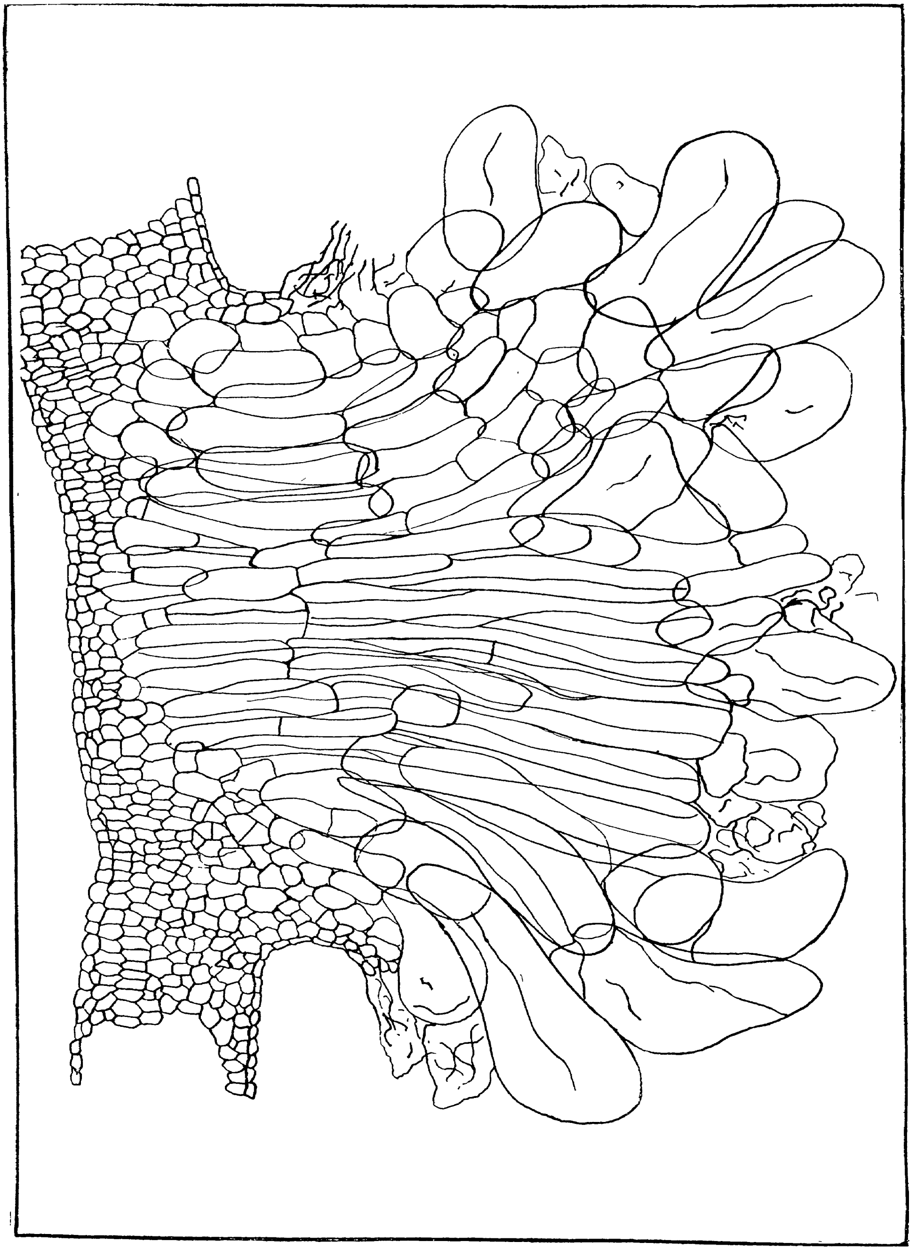
LEAF STRUCTURE OF CAULIFLOWER.



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